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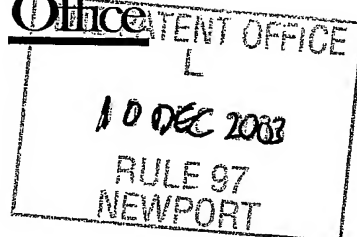
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*Ludwig Institute for Cancer Research
Postfach 8024
Zurich
SwitzerlandPatents ADP number *(if you know it)*

577155002

If the applicant is a corporate body, give the country/state of its incorporation

CH

4. Title of the invention

Tumour Suppressor Protein

5. Name of your agent *(if you have one)*

Harrison Goddard Foote

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*31 St Saviourgate
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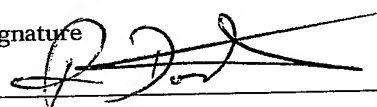
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Tumour Suppressor Protein

The invention relates to a protein which binds and modulates the activity of a tumour suppressor protein, for example p53; a nucleic acid molecule encoding said protein and screening methods which modulate the binding activity of said polypeptide for its target polypeptide.

Tumour suppressor genes encode proteins which function to inhibit cell growth or division and are therefore important with respect to maintaining proliferation, growth and differentiation of normal cells. Mutations in tumour suppressor genes result in abnormal cell-cycle progression whereby the normal cell-cycle check points which arrest the cell-cycle, when, for example, DNA is damaged, are ignored and damaged cells divide uncontrollably. The products of tumour suppressor genes function in all parts of the cell (e.g. cell surface, cytoplasm, nucleus) to prevent the passage of damaged cells through the cell-cycle (i.e. G1, S, G2, M and cytokinesis). A number of tumour suppressor genes have been isolated and sequenced. These include the Retinoblastoma gene (Rb), mutations in which are linked to cancers such as bone (osteocarcinoma), bladder, small cell lung and breast cancer, as well as retinoblastoma. The Wilms Tumour 1 gene (WT-1), mutations in which are linked to nephroblastoma and neurofibromatosis.

Arguably the tumour suppressor gene which has been the subject of the most intense research is p53. p53 encodes a protein which functions as a transcription factor and is a key regulator of the cell division cycle. It was discovered in 1978 (Lane and Crawford, 1979) as a protein shown to bind with affinity to the SV40 large T antigen. The p53 gene encodes a 393 amino acid polypeptide with a molecular weight of 53kDa. One of the most important tumour suppression functions of p53 is its ability to induce apoptosis

Apoptosis, or programmed cell death, is a process by which multi-cellular organisms regulate cell number and differentiation. The process is regulated by factors which either induce or prevent apoptosis. Inducers of apoptosis include Bcl-2 family members, caspase family members and their associated factors Apaf-1 and Fadd. Caspases are synthesised as proenzymes which become activated after proteolytic cleavage. The active caspase then induces many of the morphological and biochemical changes associated with apoptosis. Mitochondria play a pivotal role in the activation process through the release of pro-apoptotic factors such as cytochrome c, AIF and Diablo. The release from mitochondria is controlled by the Bcl-2 family of proteins; (e.g. Bcl-2 and Bcl-xl inhibit release; Bax and Bak induce release).

The polypeptide referred to as iASPP that is described in WO02/12325 is a further example of an agent involved in the regulation of apoptosis.

We describe a variant iASPP polypeptide which has characteristics which are distinct from those described in W02/12325. The polypeptide, referred to as iASPP 6C, is extended at its amino terminus by **X amino acid residues** and binds preferentially to p53 when compared to iASPP. iASPP C6 preferentially binds p53 when compared to the shorter version described in W02/12325. The shorter version preferentially binds the apoptosis inducer protein Bcl 2.

iASPP C6 is a ubiquitinated polypeptide which likely controls the turnover of iASPP C6 *in vivo*. Ubiquitin is a small protein made up of 76 amino acids which is highly conserved across species. The most important function assigned to ubiquitin is in regulating protein turnover. Research in recent years has identified many accessory proteins involved in ubiquitin induced proteolysis. The first step is the ligation of ubiquitin to a target protein which is destined for degradation. This is mediated by three proteins referred to as E1, E2 and E3. Ubiquitin is first activated by E1 activating enzyme, a homodimer composed of two identical 105kDa subunits which is ligated to ubiquitin via a

thioester bond. Following activation the E1:ubiquitin conjugate is transported by E2 (referred to as a carrier protein). The E2 proteins vary markedly in size but do have some conserved elements. The E2 protein accepts the ubiquitin from E1 and forms a second complex again via a thioester bond. The E3
5 protein may or may not become involved in the final step, which is the transfer of ubiquitin to a protein substrate. This is followed by recognition by a protease, which degrades the ubiquitinated protein. The protease may be part of a structure referred to as the proteasome which is a large multi-subunit complex of proteases and associated co-factors. In some examples proteins can
10 become polyubiquitinated, which results from ubiquitin proteins being ligated to ubiquitin proteins, which are already ligated to a target protein.

According to an aspect of the invention there is provided an isolated polypeptide wherein said polypeptide is represented by the amino acid
15 sequence as shown in Figure 1a, or a variant polypeptide which variant is modified by addition, deletion or substitution of at least one amino acid residue characterised in that said polypeptide has the following characteristics:

- i) a polypeptide which preferentially binds the tumour suppressor polypeptide p53 to inhibit the pro-apoptotic activity of p53
20 when compared to a polypeptide, or variant thereof, as represented by the amino acid sequence as shown in Figure 2a;
- ii) a polypeptide which includes at least one amino acid residue which residue is ubiquitinated; and
- iii) a polypeptide which comprises an amino-terminal polypeptide
25 domain wherein said domain is represented between amino acid 1 and 483 of the amino acid sequence shown in Figure 1a.

In a preferred embodiment of the invention said polypeptide preferentially binds p53 when compared to a polypeptide represented by the amino acid
30 sequence shown in Figure 2a.

In a further preferred embodiment of the invention said polypeptide is modified by addition, deletion or substitution of at least one amino acid residue wherein said modification is between amino acid residues +1 and +483 of the amino acid sequence presented in Figure 1a.

5

Assays to determine the binding of polypeptides, which are herein disclosed, to for example, p53 are known in the art and described in the present application.

10 In a further preferred embodiment of the invention said polypeptide comprises the amino acid sequence shown in Figure 1a. Preferably said polypeptide consists of the amino acid sequence shown in Figure 1a.

15 According to an aspect of the invention there is provided an isolated nucleic acid molecule wherein said nucleic acid molecule encodes a polypeptide according to the invention.

20 In a preferred embodiment of the invention said nucleic acid molecule is represented by the nucleic acid sequence shown in Figure 1b or a nucleic acid molecule which hybridises to the sequence shown in Figure 1b under stringent hybridisation conditions and which encodes a polypeptide according to the invention.

25 In a preferred embodiment of the invention said nucleic acid molecule consists of the nucleic acid sequence shown in Figure 1b.

In a further preferred embodiment of the invention said isolated nucleic acid molecule is a cDNA. In an alternative preferred embodiment of the invention said nucleic acid molecule is genomic DNA.

30 According to a further aspect of the invention there is provided a vector comprising a nucleic acid molecule according to the invention. Preferably said

vector is an expression vector adapted for recombinant expression of said polypeptide.

5 Preferably, said vector is adapted for prokaryotic gene expression. In an alternative embodiment of the invention said vector is adapted for eukaryotic gene expression.

Typically said adaptation includes, by example and not by way of limitation, the provision of transcription control sequences (promoter sequences) which
10 mediate cell/tissue specific expression. These promoter sequences may be cell/tissue specific, inducible or constitutive.

Promoter is an art recognised term and includes the following features which are provided by example only, and not by way of limitation. Enhancer elements
15 are *cis* acting nucleic acid sequences often found 5' to the transcription initiation site of a gene (enhancers can also be found 3' to a gene sequence or even located in intronic sequences and is therefore position independent). Enhancers function to increase the rate of transcription of the gene to which the enhancer is linked. Enhancer activity is responsive to *trans* acting transcription
20 factors (polypeptides) which have been shown to bind specifically to enhancer elements. The binding/activity of transcription factors (please see Eukaryotic Transcription Factors, by David S Latchman, Academic Press Ltd, San Diego) is responsive to a number of environmental cues which include, by example and not by way of limitation, intermediary metabolites or environmental
25 effectors, for example temperature.

Promoter elements also include so-called TATA box and RNA polymerase initiation selection (RIS) sequences which function to select a site of transcription initiation. These sequences also bind polypeptides which function,
30 *inter alia*, to facilitate transcription initiation selection by RNA polymerase.

Adaptations also include the provision of selectable markers and autonomous replication sequences which both facilitate the maintenance of said vector in either the eukaryotic cell or prokaryotic host. Vectors which are maintained autonomously are referred to as episomal vectors. Episomal
5 vectors are desirable since these molecules can incorporate large DNA fragments (30-50kb DNA). Episomal vectors of this type are described in WO98/07876.

Adaptations which facilitate the expression of vector encoded genes include the
10 provision of transcription termination/polyadenylation sequences. This also includes the provision of internal ribosome entry sites (IRES) which function to maximise expression of vector encoded genes arranged in bicistronic or multicistronic expression cassettes.

15 These adaptations are well known in the art. There is a significant amount of published literature with respect to expression vector construction and recombinant DNA techniques in general. Please see, Sambrook et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY and references therein; Marston, F (1987) DNA
20 Cloning Techniques: A Practical Approach Vol III IRL Press, Oxford UK; DNA Cloning: F M Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons, Inc.(1994).

According to a fourth aspect of the invention there is provided a method for the production of the polypeptide according to the invention comprising:

- 25 i) providing a cell transformed/transfected with a nucleic acid molecule according to the invention;
- ii) growing said cell in conditions conducive to the manufacture of said polypeptide; and
- i) purifying said polypeptide from said cell, or its growth environment.

In a preferred embodiment of the invention said nucleic acid molecule is the vector according to the invention.

In a preferred method of the invention said vector encodes, and thus said recombinant polypeptide is provided with, a secretion signal to facilitate
5 purification of said polypeptide.

According to a further aspect of the invention there is provided an antibody which binds the polypeptide according to the invention characterised in that said antibody binds said polypeptide between amino acid residues +1 to +483
10 of the amino acid sequence shown in Figure 1a.

Preferably said antibody does not bind said polypeptide represented by the sequence +484 to +828 of the amino acid sequence shown in Figure 1a.

15 Antibodies, also known as immunoglobulins, are protein molecules which usually have specificity for foreign molecules (antigens). Immunoglobulins (Ig) are a class of structurally related proteins consisting of two pairs of polypeptide chains, one pair of light (L) (low molecular weight) chain (κ or λ), and one pair of heavy (H) chains (γ , α , μ , δ and ϵ), all four linked together by
20 disulphide bonds. Both H and L chains have regions that contribute to the binding of antigen and that are highly variable from one Ig molecule to another. In addition, H and L chains contain regions that are non-variable or constant.

The L chains consist of two domains. The carboxy-terminal domain is
25 essentially identical among L chains of a given type and is referred to as the "constant" (C) region. The amino terminal domain varies from L chain to L chain and contributes to the binding site of the antibody. Because of its variability, it is referred to as the "variable" (V) region.

30 The H chains of Ig molecules are of several classes, α , μ , σ , α , and γ (of which there are several sub-classes). An assembled Ig molecule consisting of one or

more units of two identical H and L chains, derives its name from the H chain that it possesses. Thus, there are five Ig isotypes: IgA, IgM, IgD, IgE and IgG (with four sub-classes based on the differences in the 'constant' regions of the H chains, i.e., IgG1, IgG2, IgG3 and IgG4). Further detail regarding antibody
5 structure and their various functions can be found in, Using Antibodies: A laboratory manual, Cold Spring Harbour Laboratory Press.

In a preferred embodiment of the invention said fragment is a Fab fragment.

10 In a further preferred embodiment of the invention said antibody is selected from the group consisting of: F(ab')₂, Fab, Fv and Fd fragments; and antibodies comprising CDR3 regions.

Preferably said fragments are single chain antibody variable regions (scFV's) or
15 domain antibodies. If a hybridoma exists for a specific monoclonal antibody it is well within the knowledge of the skilled person to isolate scFv's from mRNA extracted from said hybridoma via RT PCR. Alternatively, phage display screening can be undertaken to identify clones expressing scFv's. Domain antibodies are the smallest binding part of an antibody (approximately 13kDa).
20 Examples of this technology is disclosed in US6, 248, 516, US6, 291, 158, US6,127, 197 and EP0368684 which are all incorporated by reference in their entirety.

A modified antibody, or variant antibody and reference antibody, may differ in
25 amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination. Among preferred variants are those that vary from a reference polypeptide by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid by another amino acid of like characteristics. The following non-limiting
30 list of amino acids are considered conservative replacements (similar): a) alanine, serine, and threonine; b) glutamic acid and aspartic acid; c)

asparagine and glutamine d) arginine and lysine; e) isoleucine, leucine, methionine and valine and f) phenylalanine, tyrosine and tryptophan. Most highly preferred are variants which show enhanced biological activity.

5 Preferably said antibody is a humanised or chimeric antibody.

A chimeric antibody is produced by recombinant methods to contain the variable region of an antibody with an invariant or constant region of a human antibody.

10

A humanised antibody is produced by recombinant methods to combine the complementarity determining regions (CDRs) of an antibody with both the constant (C) regions and the framework regions from the variable (V) regions of a human antibody.

15

Chimeric antibodies are recombinant antibodies in which all of the V-regions of a mouse or rat antibody are combined with human antibody C-regions. Humanised antibodies are recombinant hybrid antibodies which fuse the complementarity determining regions from a rodent antibody V-region with the framework regions from the human antibody V-regions. The C-regions from the human antibody are also used. The complementarity determining regions (CDRs) are the regions within the N-terminal domain of both the heavy and light chain of the antibody to where the majority of the variation of the V-region is restricted. These regions form loops at the surface of the antibody molecule. These loops provide the binding surface between the antibody and antigen.

20
25

Antibodies from non-human animals provoke an immune response to the foreign antibody and its removal from the circulation. Both chimeric and humanised antibodies have reduced antigenicity when injected to a human subject because there is a reduced amount of rodent (i.e. foreign) antibody

30

within the recombinant hybrid antibody, while the human antibody regions do not elicit an immune response. This results in a weaker immune response and a decrease in the clearance of the antibody. This is clearly desirable when using therapeutic antibodies in the treatment of human diseases. Humanised antibodies are designed to have less "foreign" antibody regions and are therefore thought to be less immunogenic than chimeric antibodies.

According to a further aspect of the invention the invention there is provided a polypeptide according to the invention for use as a pharmaceutical.

According to a further aspect of the invention there is provided a nucleic acid according to the invention for use as a pharmaceutical.

In a preferred embodiment of the invention said pharmaceutical further comprises a diluent, carrier or excipient.

When administered, the therapeutic compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents, such as chemotherapeutic agents.

The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When antibodies are used therapeutically, a preferred route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the

antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing antibody
5 aerosols without resort to undue experimentation. When using antisense preparations of the invention, slow intravenous administration is preferred.

The compositions of the invention are administered in effective amounts. An "effective amount" is that amount of a composition that alone, or together with
10 further doses, produces the desired response. In the case of treating a particular disease, such as cancer, the desired response is inhibiting the progression of the disease. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be
15 monitored according to diagnostic methods of the invention discussed herein.

Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of
20 concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the
25 highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

30 The pharmaceutical compositions used in the foregoing methods preferably are sterile and contain an effective amount of for example, a dominant negative

iASPP C6 or nucleic acid encoding a dominant negative iASPP C6, for producing the desired response in a unit of weight or volume suitable for administration to a patient. The response can, for example, be measured by determining the signal transduction inhibited by the dominant negative iASPP C6, composition via a reporter system, by measuring downstream effects such as gene expression, or by measuring the physiological effects of the iASPP C6 composition, such as regression of a tumour, decrease of disease symptoms, modulation of apoptosis, etc.

The doses of dominant negative iASPP C6 polypeptide or nucleic acid administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

In general, doses of dominant negative iASPP C6 are formulated and administered in doses between 1 ng and about 500mg, and between 10 ng and 100mg, according to any standard procedure in the art. Where nucleic acids encoding dominant negative iASPP C6 are employed, doses of between 1 ng and 0.1mg generally will be formulated and administered according to standard procedures. Other protocols for the administration of iASPP C6 compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of injections, sites of injections, mode of administration (e.g., intra-tumoral) and the like vary from the foregoing. Administration of iASPP C6 compositions to mammals other than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out under substantially the same conditions as described above. A subject, as used herein, is a mammal,

preferably a human, and including a non-human primate, cow, horse, pig, sheep, goat, dog, cat or rodent.

When administered, the pharmaceutical preparations of the invention are
5 applied in pharmaceutically-acceptable amounts and in pharmaceutically-
acceptable compositions. The term "pharmaceutically acceptable" means a
non-toxic material that does not interfere with the effectiveness of the
biological activity of the active ingredients. Such preparations may routinely
contain salts, buffering agents, preservatives, compatible carriers, and
10 optionally other therapeutic agents. When used in medicine, the salts should be
pharmaceutically acceptable, but non-pharmaceutically acceptable salts may
conveniently be used to prepare pharmaceutically-acceptable salts thereof and
are not excluded from the scope of the invention. Such pharmacologically and
pharmaceutically-acceptable salts include, but are not limited to, those
15 prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric,
phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the
like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal
or alkaline earth salts, such as sodium, potassium or calcium salts.

20 iASPP C6 compositions may be combined, if desired, with a pharmaceutically-
acceptable carrier. The term "pharmaceutically-acceptable carrier" as used
herein means one or more compatible solid or liquid fillers, diluents or
encapsulating substances which are suitable for administration into a human.
The term "carrier" denotes an organic or inorganic ingredient, natural or
25 synthetic, with which the active ingredient is combined to facilitate the
application. The components of the pharmaceutical compositions also are
capable of being co-mingled with the molecules of the present invention, and
with each other, in a manner such that there is no interaction which would
substantially impair the desired pharmaceutical efficacy.

30

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

- 5 The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

10 The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided
15 solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in
20 aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of iASPP C6 polypeptides or nucleic acids, which is preferably isotonic with the blood of the recipient. This
25 preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be
30 employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or

suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

In a preferred embodiment of the invention said nucleic acid molecule is an inhibitory RNA (RNAi) molecule or antisense nucleic acid molecule.

In a preferred embodiment of the invention said nucleic acid molecule is selected from the group consisting of an antisense molecule or an inhibitory RNA molecule designed with reference to the nucleic acid sequence shown in Figure 1b. Preferably said antisense or inhibitory RNA molecule is designed to that part of said nucleic acid sequence which encodes an amino acid sequence as defined by amino acid residues +1 to +483 as shown in Figure 1a.

As used herein, the term "antisense molecule" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridises under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridisation with the target gene or transcript. Those skilled in the art will recognise that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridise substantially more to the target

sequence than to any other sequence in the target cell under physiological conditions. Based upon the iASPP 6C nucleic acid sequences provided herein, or upon allelic or homologous genomic and/or cDNA sequences, one of skill in the art can easily choose and synthesise any of a number of appropriate antisense molecules for use in accordance with the present invention. For example, a "gene walk" comprising a series of oligonucleotides of 15-30 nucleotides spanning the length of iASPP 6C nucleic acid can be prepared, followed by testing for inhibition of the corresponding iASPP 6C expression. Optionally, gaps of 5-10 nucleotides can be left between the oligonucleotides to reduce the number of oligonucleotides synthesised and tested.

In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides (Wagner et al., *Nature Biotechnol.* 14:840-844, 1996). Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which proteins are not expected to bind. Finally, although iASPP 6C cDNA sequences are disclosed herein, one of ordinary skill in the art may easily derive the genomic DNA corresponding to the cDNAs. Thus, the present invention also provides for antisense oligonucleotides which are complementary to

iASPP 6C genomic DNA. Similarly, antisense to allelic or homologous cDNAs and genomic DNAs are enabled without undue experimentation.

5 In one set of embodiments, the antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognised methods which may be carried out manually or by
10 an automated synthesiser. They also may be produced recombinantly by vectors.

In a preferred embodiment of the invention there is provided a transcription cassette comprising an nucleic acid sequence operatively linked to a promoter
15 which promoter transcribes said nucleic acid molecule to produce an antisense nucleic acid molecule, said sequence selected from the group consisting of:

- i) a nucleic acid sequence, or part thereof, as represented in Figure 1b;
- ii) a nucleic acid sequence which hybridises to the sense sequence
20 presented in Figure 1b and which encodes a polypeptide according to the invention.

A recent technique to specifically ablate gene function is through the introduction of double stranded RNA, also referred to as inhibitory RNA
25 (RNAi), into a cell which results in the destruction of mRNA complementary to the sequence included in the RNAi molecule. The RNAi molecule comprises two complementary strands of RNA (a sense strand and an antisense strand) annealed to each other to form a double stranded RNA molecule. The RNAi molecule is typically derived from exonic or coding sequence of the
30 gene which is to be ablated.

Recent studies suggest that RNAi molecules ranging from 100-1000bp derived from coding sequence are effective inhibitors of gene expression. Surprisingly, only a few molecules of RNAi are required to block gene expression which implies the mechanism is catalytic. The site of action appears to be nuclear as
5 little if any RNAi is detectable in the cytoplasm of cells indicating that RNAi exerts its effect during mRNA synthesis or processing.

In a further preferred embodiment of the invention there is provided a transcription cassette comprising a nucleic acid molecule, or part thereof,
10 selected from the group consisting of:

- i) a nucleic acid molecule represented by the nucleic acid sequence in Figure 1b;
- ii) a nucleic acid molecule which hybridises to the sequence in (i) above and which encodes a polypeptide according to the
15 invention; or
- iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said
20 cassette.

In a preferred embodiment of the invention said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

25

In a further preferred embodiment of the invention said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid
30 molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

In a preferred embodiment of the invention said first and second parts are linked by at least one nucleotide base.

- 5 In a preferred embodiment of the invention said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 nucleotide bases.

In a further preferred embodiment of the invention the length of the RNAi molecule is between 100bp-1000bp. More preferably still the length of RNAi
10 is selected from 100bp; 200bp; 300bp; 400bp; 500bp; 600bp; 700bp; 800bp; 900bp; or 1000bp. More preferably still said RNAi is at least 1000bp.

In an alternative preferred embodiment of the invention the RNAi molecule is between 15bp and 25bp, preferably said molecule is 21bp.

15

In a preferred embodiment of the invention said cassette is part of a vector.

According to a further aspect of the invention there is provided a screening method to identify an agent which modulates the interaction of p53 binding
20 proteins with a p53 polypeptide wherein said method comprises the following steps of:

- i) forming a preparation comprising a polypeptide according to the invention and a p53 polypeptide, or sequence variant thereof, and at least on agent to be tested;
- 25 ii) determining the activity of said agent with respect to the binding of said polypeptide to p53 polypeptide.

According to a further aspect of the invention there is provided a screening method for the identification of an agent which modulates the interaction of
30 Bcl-2 binding polypeptides with a Bcl-2 polypeptide wherein said method comprises the steps of:

- i) forming a preparation comprising a polypeptide as represented by the amino acid sequence shown in Figure 2a, or a variant polypeptide which is modified by addition deletion or substitution of at least one amino acid residue and a Bcl-2 polypeptide or variant thereof, and at least one agent to be tested; and
- ii) determining the activity of said agent with respect to the binding of said polypeptide for said Bcl-2 polypeptide.

10 According to a yet further aspect of the invention there is provided a screening method to identify agents which modulate the ubiquitination of a polypeptide according to the invention comprising the steps of:

- i) forming a preparation comprising a polypeptide according to the invention, a ubiquitin polypeptide or variant thereof, polypeptide(s) with the specific activity associated with ubiquitin conjugating polypeptides and at least one agent to be tested;
- ii) determining the activity of said agent with respect to the conjugation of ubiquitin to said polypeptide.

20 In a preferred method of the invention said agent is a peptide or polypeptide.

In a preferred method of the invention said peptide is at least 6 amino acid residues in length. Preferably the length of said peptide/polypeptide is selected from the group consisting of: at least 7 amino acid residues; 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues in length. Alternatively the length of said peptide/polypeptide is at least 20 amino acid residues; 30; 40; 50; 60; 70; 80; 90; or 100 amino acid residues in length.

30 It will be apparent to one skilled in the art that modification to the amino acid sequence of peptides agents could enhance the binding and/or stability of the

peptide with respect to its target sequence. In addition, modification of the peptide may also increase the *in vivo* stability of the peptide thereby reducing the effective amount of peptide necessary to inhibit p53 binding of iASPP. This would advantageously reduce undesirable side effects which may result in vivo. Modifications include, by example and not by way of limitation, acetylation and amidation. Alternatively or preferably, said modification includes the use of modified amino acids in the production of recombinant or synthetic forms of peptides. It will be apparent to one skilled in the art that modified amino acids include, by way of example and not by way of limitation, 4-hydroxyproline, 5-hydroxylysine, N⁶-acetyllysine, N⁶-methyllysine, N⁶,N⁶-dimethyllysine, N⁶,N⁶,N⁶-trimethyllysine, cyclohexylalanine, D-amino acids, ornithine. Other modifications include amino acids with a C₂, C₃ or C₄ alkyl R group optionally substituted by 1, 2 or 3 substituents selected from halo (eg F, Br, I), hydroxy or C₁-C₄ alkoxy. Modifications also include, by example and not by way of limitation, acetylation and amidation.

In a preferred embodiment of the invention said peptide sequence is acetylated. Preferably said acetylation is to the amino terminus of said peptide.

In a further preferred embodiment of the invention said peptide sequence is amidated. Preferably said amidation is to the carboxyl-terminus of said peptide.

It will also be apparent to one skilled in the art that peptides could be modified by cyclisation. Cyclisation is known in the art, (see Scott *et al* Chem Biol (2001), 8:801-815; Gellerman *et al* J. Peptide Res (2001), 57: 277-291; Dutta *et al* J. Peptide Res (2000), 8: 398-412; Ngoka and Gross J Amer Soc Mass Spec (1999), 10:360-363.

In a further preferred method of the invention said antagonist is an antibody or antibody binding part. Preferably said antibody is a monoclonal antibody or binding part thereof.

- 5 In an alternative preferred method of the invention said agent is an aptamer.

Nucleic acids have both linear sequence structure and a three dimensional structure which in part is determined by the linear sequence and also the environment in which these molecules are located. Conventional therapeutic
10 molecules are small molecules, for example, peptides, polypeptides, or antibodies, which bind target molecules to produce an agonistic or antagonistic effect. It has become apparent that nucleic acid molecules also have potential with respect to providing agents with the requisite binding properties which may have therapeutic utility. These nucleic acid molecules are typically referred
15 to as aptamers. Aptamers are small, usually stabilised, nucleic acid molecules which comprise a binding domain for a target molecule. A screening method to identify aptamers is described in US 5,270,163 which is incorporated by reference. Aptamers are typically oligonucleotides which may be single stranded oligodeoxynucleotides, oligoribonucleotides, or modified
20 oligodeoxynucleotide or oligoribonucleotides.

The term "modified" encompasses nucleotides with a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars which are covalently attached to low molecular weight organic groups
25 other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified nucleotides may also include 2' substituted sugars such as 2'-O-methyl-; 2'-O-alkyl; 2'-O-allyl; 2'-S-alkyl; 2'-S-allyl; 2'-fluoro-; 2'-halo or 2'-azido-ribose, carbocyclic sugar analogues a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars,
30 furanose sugars, and sedoheptulose.

- Modified nucleotides are known in the art and include by example and not by way of limitation; alkylated purines and/or pyrimidines; acylated purines and/or pyrimidines; or other heterocycles. These classes of pyrimidines and purines are known in the art and include, pseudoisocytosine; N4, N4-ethanocytosine; 8-hydroxy-N6-methyladenine; 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil; 5-fluorouracil; 5-bromouracil; 5-carboxymethylaminomethyl-2-thiouracil; 5-carboxymethylaminomethyl uracil; dihydrouracil; inosine; N6-isopentyl-adenine; 1-methyladenine; 1-methylpseudouracil; 1-methylguanine; 2,2-dimethylguanine; 2-methyladenine; 2-methylguanine; 3-methylcytosine; 5-methylcytosine; N6-methyladenine; 7-methylguanine; 5-methylaminomethyl uracil; 5-methoxy amino methyl-2-thiouracil; β -D-mannosylqueosine; 5-methoxycarbonylmethyluracil; 5-methoxyuracil; 2-methylthio-N6-isopentenyladenine; uracil-5-oxyacetic acid methyl ester; pseudouracil; 2-thiocytosine; 5-methyl-2-thiouracil, 2-thiouracil; 4-thiouracil; 5-methyluracil; N-uracil-5-oxyacetic acid methylester; uracil 5-oxyacetic acid; queosine; 2-thiocytosine; 5-propyluracil; 5-propylcytosine; 5-ethyluracil; 5-ethylcytosine; 5-butyluracil; 5-pentyluracil; 5-pentylcytosine; and 2,6-diaminopurine; methylpseudouracil; 1-methylguanine; 1-methylcytosine.
- The aptamers of the invention are synthesized using conventional phosphodiester linked nucleotides and synthesized using standard solid or solution phase synthesis techniques which are known in the art. Linkages between nucleotides may use alternative linking molecules. For example, linking groups of the formula $P(O)S$, (thioate); $P(S)S$, (dithioate); $P(O)NR_2$; $P(O)R$; $P(O)OR_6$; CO ; or $CONR_2$ wherein R is H (or a salt) or alkyl (1-12C) and R_6 is alkyl (1-9C) is joined to adjacent nucleotides through $-O-$ or $-S-$. The binding of aptamers to a target polypeptide is readily tested by assays hereindisclosed.
- An embodiment of the invention will now be described by example only and with reference to the following figures:

Figure 1a is the amino acid sequence of iASPP C6 protein, amino acid sequence underlined is identical to iASPP; Figure 1b is the nucleic acid sequence of iASPP6C;

5

Figure 2a is the amino acid sequence of iASPP; Figure 2b is the nucleic acid sequence of iASPP;

Figure 3 is a sequence alignment of full length iASPP6C and iASPP;

10

Figure 4 illustrates the expression of iASPP expression in various cell lines. Full length iASPP 6C is detected at about 100kDa. The samples probed with antibody LX049.3 are: 2.1kb iASPP/RAI (truncated iASPP) in vitro translated; RKO (colon cancer cell line); HeLa; 293 (kidney); MCF7 (breast); SaOS2 (osteosarcoma); HBL100 (breast); H1299 (lung); U937 (lung); U2OS (osteosarcoma);

15

Figure 5 illustrates an immunoprecipitation/western blot using two different iASPP antibodies: LX049.3 is a mouse monoclonal while pAb 18 is a rabbit antibody (epitopes are given in the peptide alignment, see Figure 3);

20

Figure 6a and 6b illustrates that iASPP 6C is ubiquitinated. This process results in the generation of the 83kDa fragment, which is abolished in the presence of MG132 (ubiquitin-proteasome inhibitor). Ubiquitination also appears to be dependent on cell density. The cells were split according the cell density required and MG132 was added the next day (16-24 hrs later).

25

Figure 7 illustrates that p53 preferentially binds to full length iASPP6C, while Bcl-2 preferentially binds iASPP;

30

Figure 8 illustrates the activity of the full length iASPP6C in cells and that iASPP and p53 are involved in the activation of apoptotic genes but not cell cycle regulatory genes, and that it also interacts with p63 and p73; and

- 5 Figure 9 illustrates that the full length iASPP6C is preferentially expressed in cells rather than iASPP but at different expression levels.

Materials and Methods

- 10 Standard molecular and cell biology methods have been used in the exemplification of the invention hereindisclosed and can be readily found in published literature. For example, molecular biological techniques can be found in Sambrook et al Molecular Cloning, A Laboratory Manual, Second Edition (Cold Spring Harbor Laboratory Press 1989); immunological
15 techniques used herein can be found in Using Antibodies, A Laboratory Manual, (Cold Spring Harbor Laboratory Press 1999).

20

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Claims

1. An isolated polypeptide wherein said polypeptide is represented by the amino acid sequence as shown in Figure 1a, or a variant polypeptide which variant is modified by addition, deletion or substitution of at least one amino acid residue characterised in that said polypeptide has the following characteristics:
- i) a polypeptide which preferentially binds the tumour suppressor polypeptide p53 to inhibit the pro-apoptotic activity of p53 when compared to a polypeptide, or variant thereof, as represented by the amino acid sequence as shown in Figure 2a;
 - ii) a polypeptide which includes at least one amino acid residue which residue is ubiquitinated; and
 - iii) a polypeptide which comprises an amino-terminal polypeptide domain wherein said domain is represented between amino acid 1 and 483 of the amino acid sequence shown in Figure 1a.
2. A polypeptide according to Claim 1 wherein said polypeptide preferentially binds p53 when compared to a polypeptide represented by the amino acid sequence shown in Figure 2a.
3. A polypeptide according to Claim 1 or 2 wherein said polypeptide is modified by addition, deletion or substitution of at least one amino acid residue wherein said modification is between amino acid residues 1 and 483 of the amino acid sequence presented in Figure 1a.
4. A polypeptide according to any of Claims 1-3 wherein said polypeptide comprises the amino acid sequence shown in Figure 1a.
5. A polypeptide according to any of Claims 1-4 wherein said polypeptide consists of the amino acid sequence shown in Figure 1a.

6. An isolated nucleic acid molecule wherein said nucleic acid molecule encodes a polypeptide according to any of Claims 1-5.
- 5 7. An isolated nucleic acid molecule according to Claim 6 wherein said nucleic acid molecule is represented by the nucleic acid sequence shown in Figure 1b or a nucleic acid molecule which hybridises to the sequence shown in Figure 1b, under stringent hybridisation conditions, and which encodes a polypeptide according to any of Claims 1-5.
- 10 8. A nucleic acid molecule according to Claim 6 or 7 wherein said nucleic acid molecule consists of the nucleic acid sequence shown in Figure 1b.
- 15 9. A nucleic acid molecule according to any of Claims 6-8 wherein said molecule is a cDNA.
10. A nucleic acid molecule according to any of Claims 6-8 wherein said molecule is genomic DNA.
- 20 11. A vector comprising a nucleic acid molecule according to any of Claims 6-10.
12. A method for the production of the polypeptide according to any of Claims 1-5, comprising the steps:
- 25 i) providing a cell transformed/transfected with a nucleic acid molecule or vector according to any of Claims 6-11;
- ii) growing said cell in conditions conducive to the manufacture of said polypeptide; and
- iii) purifying said polypeptide from said cell, or its growth environment.

13. An antibody, or binding fragment thereof, which binds the polypeptide according to any of Claims 1-5 characterised in that said antibody binds said polypeptide between amino acid residues 1 to 483 of the amino acid sequence shown in Figure 1a.
- 5
14. An antibody according to Claim 13 wherein said fragment is a Fab fragment.
15. An antibody fragment according to Claim 14 wherein said antibody is selected from the group consisting of: F(ab')₂, Fab, Fv and Fd fragments; and antibodies comprising CDR3 regions.
- 10
16. An antibody, or binding fragment thereof, according to any of Claims 13-15 wherein said antibody is a humanised.
- 15
17. An antibody, or binding fragment thereof, according to any of Claims 13-15 wherein said antibody is a chimeric antibody.
18. A polypeptide according to any of Claims 1-5 for use as a pharmaceutical.
- 20
19. A nucleic acid molecule or vector according to any of Claims 6-11 for use as a pharmaceutical.
- 25
20. Use according to Claim 19 wherein said nucleic acid molecule is an inhibitory RNA molecule.
21. Use according to Claim 19 wherein said nucleic acid molecule is an antisense nucleic acid molecule.
- 30

22. Use according to Claim 20 or 21 wherein said nucleic acid molecule is selected from the group consisting of an antisense molecule or an inhibitory RNA molecule designed with reference to the nucleic acid sequence shown in Figure 3, wherein said antisense or inhibitory RNA molecule is designed to that
5 part of said nucleic acid sequence which encodes amino acid residue 1 to 483 defined as shown in Figure 1a.

23. Use according to Claim 22 wherein said nucleic acid molecule is provided as a transcription cassette comprising an nucleic acid sequence
10 operatively linked to a promoter which promoter transcribes said nucleic acid molecule to produce an antisense nucleic acid molecule, said sequence selected from the group consisting of:

- i) a nucleic acid sequence, or part thereof, as represented in Figure 1b;
- 15 ii) a nucleic acid sequence which hybridises to the sense sequence presented in Figure 1b and which encodes a polypeptide according any of Claims 1-6.

24. Use according to Claim 22 wherein said nucleic acid molecule is provided as a transcription cassette comprising a nucleic acid molecule, or part thereof, selected from the group consisting of:

- i) a nucleic acid molecule represented by the nucleic acid sequence in Figure 1b;
- 25 ii) a nucleic acid molecule which hybridises to the sequence in (i) above and which encodes a polypeptide according to any of Claims 1-5; or
- 30 iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

25. Use according to Claim 24 wherein said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

5

26. Use according to Claim 24 wherein said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

10

27. Use according to Claim 26 wherein said first and second parts are linked by at least one nucleotide base.

15

28. Use according to any of Claims 23-27 wherein said cassette is part of a vector.

20

29. A screening method to identify an agent which modulates the interaction of a p53 binding protein with a p53 polypeptide wherein said method comprises the following steps of:

- i) forming a preparation comprising a polypeptide according to any of Claims 1-5 and a p53 polypeptide, or sequence variant thereof, and at least one agent to be tested;
- 25 iii) determining the activity of said agent with respect to the binding of said polypeptide to said p53 polypeptide.

30

30. A screening method for the identification of an agent which modulates the interaction of a Bcl-2 binding polypeptide with a Bcl-2 polypeptide wherein said method comprises the steps of:

- 5
- i) forming a preparation comprising a polypeptide as represented by the amino acid sequence shown in Figure 2a, or a variant polypeptide which is modified by addition deletion or substitution of at least one amino acid residue and a Bcl-2 polypeptide, or variant thereof, and at least one agent to be tested; and
 - iii) determining the activity of said agent with respect to the binding of said polypeptide to said Bcl-2 polypeptide.

10 31. A screening method to identify agents which modulate the ubiquitination of a polypeptide comprising the steps of:

- 15
- i) forming a preparation comprising a polypeptide according to any of Claims 1-5, a ubiquitin polypeptide or variant thereof, polypeptide(s) with the specific activity associated with ubiquitin conjugating polypeptides and at least one agent to be tested;
 - ii) determining the activity of said agent with respect to the conjugation of ubiquitin to said polypeptide.

20 32. A method according to any of Claims 29-31 wherein said agent is a peptide or polypeptide.

25 33. A method according to Claim 32 wherein said peptide/polypeptide is an antibody or antibody binding fragment.

30 34. A method according to Claim 31 wherein said agent is an aptamer.

Abstract

Tumour Suppressor Protein

5

We describe a polypeptide which binds and modulates the activity of a tumour suppressor polypeptide, for example p53; a nucleic acid molecule encoding said protein and screening methods which modulate the binding activity of said polypeptide for its target polypeptide(s).

[illegible]

Figure 1b

CCACGCGTCCGGGAAGCCCCAGGTGCCAGGATCTGCCCGGATCCCGGCCCGCTCCGGCCCGCACCATTGGACAGCG
AGGCATTCCAGAGCGCGCGGGACTTTCTGGACATGAACATTCCAGTCGCTGGCCATGAACACATCGGATCTGAAGCA
GATGGAGCTGGACACGGCGCGGCCAACGGTTGATGAACAGCAGCTGGAGTCGCTGTGGTCAGACTCTCCCG
GCGCCTCTGTGCCCGCAGGCCGACCCCTCTTAGGCCGCCCGGTACAGCTCCAGCTCGATCCCTGAGCCCTTCG
GCAGCCGAGGGTCCCCCCGGAAGCGGCCACCGACGGCGCAGACACCCGTTCCGACGATCAGAGAGTGGCCCCAAC
CCTACACCCCTACAGCCCGTGTCCCCAACGGGACGGCCGTCGTCGCGCGCACCCCGCTTACCTGACGCGCGGAC
GCCTACGGCAGCTGGACGCGCGGACCTCGCCCGCGCCCGCGCCTTCGATGGCGCAGGCAGCTCCCTCGGCCGTG
CGCCCTCCCCGCGGCCGCGGCCAGGCCGCTCGCCAGCAGGGTCCCCCAGCGCTTTCCGACTTCTGGGCGCGCG
AGGCTCCCCCGCGGCAGCCCCCTGGCGGAGGGGCCCCAGGCCTTCTCCCCGAGCGTGGGCGCTACCGCGCCCC
CCTGCCACAGCTACGACGCGCGACCGCTCCGCTCTCGGAGCTCCCTGCTAGGCTCCGGCGGCAGCGCATTTCGCCC
CGCCTCTGCGCGCGCAAGACGACCTGACGCTGCGCGCGGCCCTCCGAAAGCCTGGAACGAGTCTGACCTGGACGT
GTGCAGCTGTTGCCTTGGAGGGGAGCAGCCTGGATGGACTGGGGGGCACCGGCAAGGACAACCTACTAGCGCCA
CCCTGCGCGCAATTACAAGGCTCTCTCTCGCCAGCGACCGGCGTTACAGACGCGGCGAGCTACCGGCGCTCGTGTG
GCTCCGCGGGGCGCTCGGGCACTTTGCTCGCAGCTGGCAGCGCGCTCAGCCGATCTTCAAGCTGCAAGACCGCTTCTGGGAG
CCAGCCCCGCGGGGCCCCGCGCCAGCGTCCCATCCCCCTCAGCATGATCTTCAAGCTGCAAGACCGCTTCTGGGAG
CACGGGGCCAGCGCGCCATGTCTCCCTGGGTCCCCCTCTTCAACCCGAGCACCCCCGCTTAAGCTGCAGCCCCAAC
CAACAACACAGCCACCAACAACAACACAGCCCCAGCTGCCCCACAGCCCCAGACCCAAACCCCAACCCCG
TACCCAGCCCCCAGCATCCCCAACAGACATGGCCCCCTGTGAACGAGCAAGACCCCAACCCCAACCCCGAGCTG
GAGCCTGAGCCGGAGATAGAGGGGCTGTGACACCAAGTCTGGAGGCTGGCGATGTGGATGAAGGCGCTGTAGCA
GGCCTCTCAGCCCCAGAGGCTCGCAGCCAGCATGCCACCGGAGGCACAGTCGGTGCCCGAGCTGGAGGAGGTGGC
ACGGGTGTTGGCGAAATTTCCCGGCCCCCTCAAACGCAAGGGCTCCTAGGACAGGCCCCCTGCTGTGGCCCTGCC
CCTACCCACAAGAAACAGTACCAGCAGATCATCAGCGCCTTCTTCATCGTATGGGGGGCCAGGGCCCGGGGGG
CGGAGCCAGAGCTGTCCCCCATCTAGGAGGATCTGAGGCCAGGGCAGGGCCCCCTGCTCTGCCCCACCAGCTCC
CATTCCACCCCGCCCCCTCCAGAGCAGCCACAGCCAGCCGACGCGCAGCATGATGCGCTCTGTGCTGCGG
AAGGCGGGCTCCCCGCGCAAGGCCCGCGCGCGCTCAACCCCTTGTGCTCTCTTGGACGCGCGCTGACCG
GGGAGCTGGAGGTGGTGCAGCAGCGGTGAAGGAGATGAACGACCCGAGCCAGCCCAACGAGGAGGGCATCTAGCC
TTGCACAACGCCATCTGCGGCGCCAACTACTCTATCGTGGATTCTCTCATCACCGCGGGTGCCAATGTCAACTCCC
CGACAGCCACCGCTGGACACCCCTTGTACTGCTGCGCGCGCTGTGCAACGACACAGTCATCTGCATGGCGCTGTGCA
CACGGCGCTGCAATCTTCGCCAACACGCTCAGCGACGGCGCCACCGCCTTCGAGAAGTGCGACCCCTTACC
GCGAGGGTTATGCTGACTGCGCCACCTACCTGGCAGACGTCGAGCAGAGTATGGGGCTGTAGAACAGCG
GGGCAGTGTACGCTCTCTGGGACTACAGCGCCGAGTTCGGGGACGAGCTGTCTTCCGCGAGGGGCGAGT
CGGTACCGTGTCTGCGGAGGACCGGGCGGAGGACCCGAGTGGTGGTGGGCGCGCTGCACGGCCAGG
AGGGCTACGTGCCGCGGAACCTACTTCGGGTGTTCCCCAGGGTGAAGCCTCAAAGGAGTAAAGTCTAGC
AGGATAGAAGGAGGTTTCTGAGGCTGACAGAAACAAGCATTCTCGCTTCCCTCCAGACCTCTCCCTCTG
TTTTTGTGCTGCCTTTATCTGCACCCCTCACCTGTCTGGTGGTGGTCTTGGCCACCGGTTCTCTGTTCTCTG
GAAGTCCAGGGAAGAAGGAGGGGCCCCAGCCTTAAATTTAGTAATCTGCCTTAGCCTTGGGAGGTCTGGG
AAGGGCTGGAAATCACTGGGGACAGGAACCACTTCTTTTGCCAAATCAGATCCCGTCCAAAGTGCCTC
CCATGCCTACCACCATCATCATCCCCAGCAAGCCACCCGCTGCCAGCGGGCTGGGATGGGGCA
CCACACCACTGGATATTCTGGGAGTCACTGCTGACACCATTCTCCGACGAGTCTTGGGCTTGGGTTG
GAAACATTGGTCTCTACCAGGATCCCTGCCCCACCTCTCCCCAATTAAGTGCCTTCACACAGCTCTGGTTT
AATGTTTATAAACAAAATAGAGAAACCTTCTTATAAATAAAAGTAGTTTGCACAGAAAAA



Figure 2a

iASPP

MWMKDPVARPLSPTRLQPALPPEAQSVPELEEVARVLAIEPRPLKRRGSMEQAPAVALPPTHKKQYQQII
SRLFHRHGGPGPGGRSQSCPPSLRDLRPGQGPLLPHQLPFHRPAPSQSSPPEQPQSMEMRSLRKAGSPR
KARRARLNPLVLLDAALTGELEVQQAVKEMNDPSQPNEEGITALHNAICGANYSIVDFLITAGANVNS
PDSHGWTPLHCAASCNDTVICMALVQHGAIFATTLSDGATAFEKCDPYREGYADCATYLADVEQSMG
LMNSGAVYALWDYSAEFGDELSFREGESVTVLRRDGPEETDWWWAALHGQEGYVPRNYFGLFPRVKP
QRSK✓

Figure 2b

1 GCGGCCGCGT CGACCCGGCG TTCAGACGCG GGCAGCTACC GGCGCTCGCT GGGCTCCGCG
61 GGGCCGTCGG GCACTTTGCC TCGCAGCTGG CAGCCCGTCA GCCGCATCCC CATGCCCCC
121 TCCAGCCCC AGCCCCGCGG GGCCCCGCGC CAGCGTCCA TCCCCCTCAG CATGATCTTC
181 AAGCTGCAGA ACGCCTTCTG GGAGCACGGG GCCAGCCGCG CCATGCTCCC TGGGTCCCC
241 CTCTTCACCC GAGCACCCCC GCCTAAGCTG CAGCCCCAAC CACAAACACA GCCCCAGCCA
301 CAATCACAAC CACAGCCCCA GTGCCCCAA CAGCCCCAGA CCAACCCCCA AACCCTACC
361 CCAGCCTCCC ACATCCGCAT CCCCACAGA CATGGCCCC TGTGAACGAA GGACCCCCA
421 AACCCCCAC CGAGCTGGAG CTTGAGCCGG AGATAGAGGG GCTGCTGACA CCAGTGCTGG
481 AGGCTGGCGA TGTGGATGAA GGACCCTGTA GCAAGGCCTC TCAGCCCCAC GAGGCTGCAG
541 CCAGCACTGC CACCGGAGGC ACAGTCGGTG CCCGAGCTGG AGGAGGTGGC ACGGGTGTG
601 GCGGAAATTC CCCGGCCCCC CAAACGCAGG GGCTCCATGG AGCAGGCCCC TGCTGTGGCC
661 CTGCCCCCTA CCCACAAGAA ACAGTACCAG CAGATCATCA GCCGCCTCTT CCATCGTCAT
721 GGGGGGCCAG GGCCCGGGG GCGGAGCCAG AGCTGTCCC CATCACTGAG GGATCTGAGG
781 CCAGGGCAGG GCCCCCTGCT CCTGCCCCAC CAGTCCCAT TCCACCGCCC GGCCCCGTCC
841 CAGAGCAGCC CACCAGAGCA GCGCAGAGC ATGGAGATGC GCTCTGTGCT GCGGAAGGCG
901 GGCTCCCCGC GCAAGGCCCG CCGCGCGCGC CTCAACCTC TGGTGCTCCT CCTGGACGCG
961 GCGCTGACCG GGGAGCTGGA GGTGGTGCA CAGGCGGTGA AGGAGATGAA CGACCCGAGC
1021 CAGCCCAACG AGGAGGGCAT CACTGCCTTG CACAACGCCA TCTGCGGCGC CAACTACTCT
1081 ATCGTGGATT TCCTCATCAC CGCGGGTGCC AATGTCAACT CCCCCGACAG CCACGGCTGG
1141 ACACCCTTGC ACTGCGCGGC GTCGTGCAAC GACACAGTCA TCTGCATGGC GCTGGTGCA
1201 CACGGCGCTG CAATCTTCGC CACCACGCTC AGCGACGGCG CCACCGCCTT CGAGAAGTGC
1261 GACCCTTACC GCGAGGGTTA TGCTGACTGC GCCACCTACC TGGCAGACGT CGAGCAGAGT
1321 ATGGGGCTGA TGAACAGCGG GGCAGTGTA GCTCTCTGGG ACTACAGCGC CGAGTTCGGG
1381 GACGAGCTGT CCTTCCGCGA GGGCGAGTCG GTCACCGTGC TGCAGAGGGA CGGGCCGGAG
1441 GAGACCGACT GGTGGTGGG CCGCTGCAC GGCCAGGAGG GCTACGTGCC GCGGAACCTAC
1501 TTCGGGCTGT TCCCCAGGT GAAGCCTCAA AGGAGTAAAG TCTAGCAGGA TAGAAGGAGG
1561 TTTCTGAGGC TGACAGAAAC AAGCATTCTT GCCTTCCCTC CAGACCTCTC CCTCTGTTTT
1621 TTGCTGCCTT TATCTGCACC CCTCACCTGT CTGGTGGTGG TCCTTGCCAC CGTTTCTCTG
1681 TTCTCTGGA AGTCCAGGGA AGAAGGAGGG CCCAGCCTT AAATTTAGTA ATCTGCCTTA
1741 GCCTTGGGAG GTCTGGGAAG GGCTGGAAAT CACTGGGAC AGGAAACCAC TTCTTTTGC
1801 CAAATCAGAT CCCGTCCAAA GTGCCTCCA TGCCTACCAC CATCATCACA TCCCCAGCA
1861 AGCCAGCCAC CTGCCAGCC GGGCCTGGGA TGGGCCACCA CCACTGGA TATTCTGGG
1921 AGTCACTGCT GACACCATCT CTCCAGCAG TCTTGGGTC TGGGTGGAA ACATTGGTCT
1981 CTACCAGGAT CCCTGCCCCA CCTCTCCCA ATTAAGTGCC TTCACACAGC ACTGGTTAA
2041 TGTTTATAAA CAAAATAGAG AACTGGTTT AATGTTATA AACAAAATAG AGAACTTTC
2101 GCTTATAAAT AAAAGTAGTT TGCACAGAAA TGAAAAAAA AAAAAAAAAA AAAAAA



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R.g3-4

JASPP GC DNA (W)
JASPP (RAI) 2.1 kb DNA
JASPP (RAI) 2.6 kb DNA

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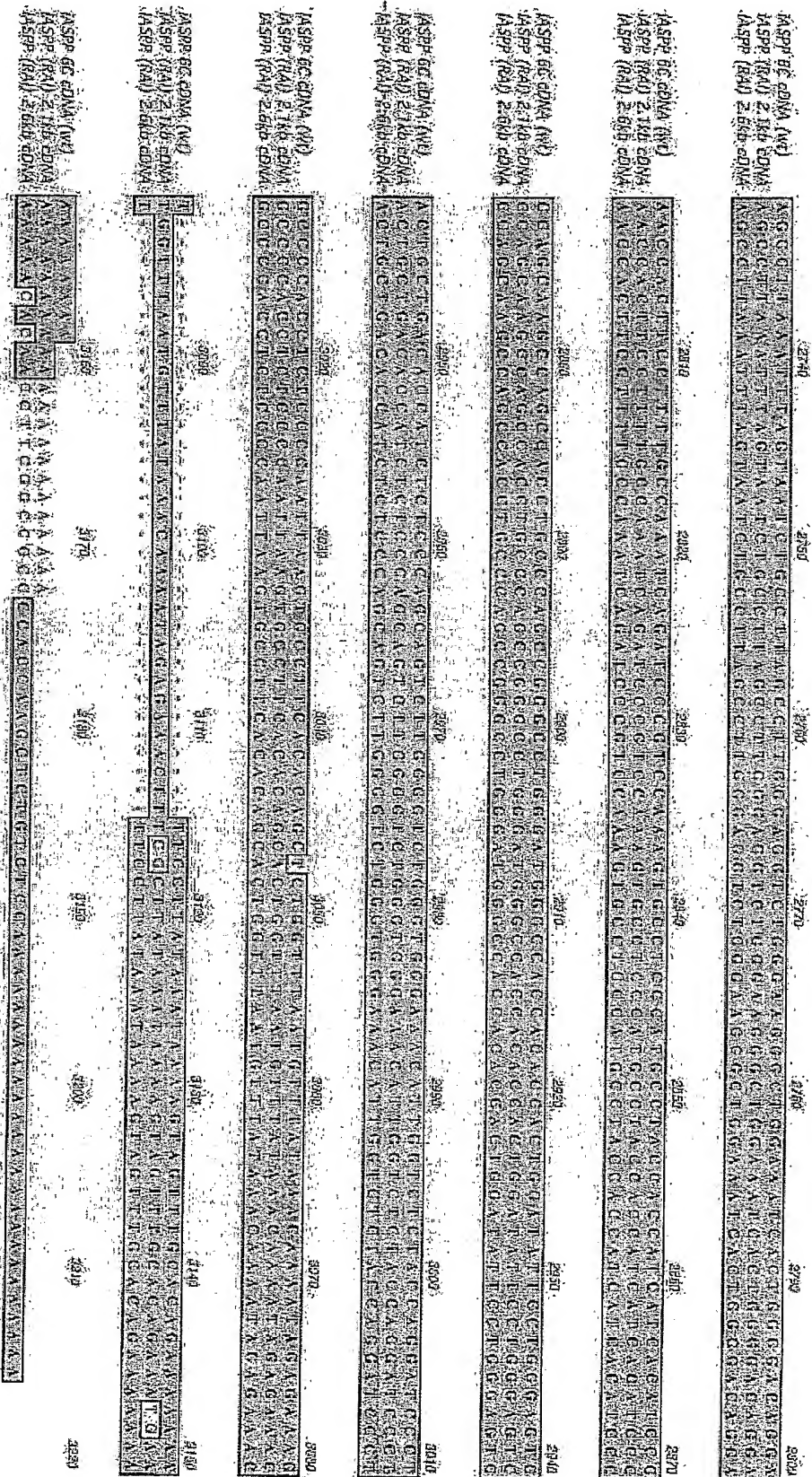
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Formatted Alignments

IASPP 60 translation
IASPP/RAI
IASPP/RAI 2.6 kb corrected TLM

MILITARY SERVICE RECORDS OF THE ARMY, NAVY, AIR FORCE, MARINE CORPS, AND COAST GUARD, 1900-1960, 1961-1969, 1970-1979, 1980-1989, 1990-1999, 2000-2009, 2010-2019, 2020-2029, 2030-2039, 2040-2049, 2050-2059, 2060-2069, 2070-2079, 2080-2089, 2090-2099, 2100-2109, 2110-2119, 2120-2129, 2130-2139, 2140-2149, 2150-2159, 2160-2169, 2170-2179, 2180-2189, 2190-2199, 2200-2209, 2210-2219, 2220-2229, 2230-2239, 2240-2249, 2250-2259, 2260-2269, 2270-2279, 2280-2289, 2290-2299, 2300-2309, 2310-2319, 2320-2329, 2330-2339, 2340-2349, 2350-2359, 2360-2369, 2370-2379, 2380-2389, 2390-2399, 2400-2409, 2410-2419, 2420-2429, 2430-2439, 2440-2449, 2450-2459, 2460-2469, 2470-2479, 2480-2489, 2490-2499, 2500-2509, 2510-2519, 2520-2529, 2530-2539, 2540-2549, 2550-2559, 2560-2569, 2570-2579, 2580-2589, 2590-2599, 2600-2609, 2610-2619, 2620-2629, 2630-2639, 2640-2649, 2650-2659, 2660-2669, 2670-2679, 2680-2689, 2690-2699, 2700-2709, 2710-2719, 2720-2729, 2730-2739, 2740-2749, 2750-2759, 2760-2769, 2770-2779, 2780-2789, 2790-2799, 2800-2809, 2810-2819, 2820-2829, 2830-2839, 2840-2849, 2850-2859, 2860-2869, 2870-2879, 2880-2889, 2890-2899, 2900-2909, 2910-2919, 2920-2929, 2930-2939, 2940-2949, 2950-2959, 2960-2969, 2970-2979, 2980-2989, 2990-2999, 3000-3009, 3010-3019, 3020-3029, 3030-3039, 3040-3049, 3050-3059, 3060-3069, 3070-3079, 3080-3089, 3090-3099, 3100-3109, 3110-3119, 3120-3129, 3130-3139, 3140-3149, 3150-3159, 3160-3169, 3170-3179, 3180-3189, 3190-3199, 3200-3209, 3210-3219, 3220-3229, 3230-3239, 3240-3249, 3250-3259, 3260-3269, 3270-3279, 3280-3289, 3290-3299, 3300-3309, 3310-3319, 3320-3329, 3330-3339, 3340-3349, 3350-3359, 3360-3369, 3370-3379, 3380-3389, 3390-3399, 3400-3409, 3410-3419, 3420-3429, 3430-3439, 3440-3449, 3450-3459, 3460-3469, 3470-3479, 3480-3489, 3490-3499, 3500-3509, 3510-3519, 3520-3529, 3530-3539, 3540-3549, 3550-3559, 3560-3569, 3570-3579, 3580-3589, 3590-3599, 3600-3609, 3610-3619, 3620-3629, 3630-3639, 3640-3649, 3650-3659, 3660-3669, 3670-3679, 3680-3689, 3690-3699, 3700-3709, 3710-3719, 3720-3729, 3730-3739, 3740-3749, 3750-3759, 3760-3769, 3770-3779, 3780-3789, 3790-3799, 3800-3809, 3810-3819, 3820-3829, 3830-3839, 3840-3849, 3850-3859, 3860-3869, 3870-3879, 3880-3889, 3890-3899, 3900-3909, 3910-3919, 3920-3929, 3930-3939, 3940-3949, 3950-3959, 3960-3969, 3970-3979, 3980-3989, 3990-3999, 4000-4009, 4010-4019, 4020-4029, 4030-4039, 4040-4049, 4050-4059, 4060-4069, 4070-4079, 4080-4089, 4090-4099, 4100-4109, 4110-4119, 4120-4129, 4130-4139, 4140-4149, 4150-4159, 4160-4169, 4170-4179, 4180-4189, 4190-4199, 4200-4209, 4210-4219, 4220-4229, 4230-4239, 4240-4249, 4250-4259, 4260-4269, 4270-4279, 4280-4289, 4290-4299, 4300-4309, 4310-4319, 4320-4329, 4330-4339, 4340-4349, 4350-4359, 4360-4369, 4370-4379, 4380-4389, 4390-4399, 4400-4409, 4410-4419, 4420-4429, 4430-4439, 4440-4449, 4450-4459, 4460-4469, 4470-4479, 4480-4489, 4490-4499, 4500-4509, 4510-4519, 4520-4529, 4530-4539, 4540-4549, 4550-4559, 4560-4569, 4570-4579, 4580-4589, 4590-4599, 4600-4609, 4610-4619, 4620-4629, 4630-4639, 4640-4649, 4650-4659, 4660-4669, 4670-4679, 4680-4689, 4690-4699, 4700-4709, 4710-4719, 4720-4729, 4730-4739, 4740-4749, 4750-4759, 4760-4769, 4770-4779, 4780-4789, 4790-4799, 4800-4809, 4810-4819, 4820-4829, 4830-4839, 4840-4849, 4850-4859, 4860-4869, 4870-4879, 4880-4889, 4890-4899, 4900-4909, 4910-4919, 4920-4929, 4930-4939, 4940-4949, 4950-4959, 4960-4969, 4970-4979, 4980-4989, 4990-4999, 5000-5009, 5010-5019, 5020-5029, 5030-5039, 5040-5049, 5050-5059, 5060-5069, 5070-5079, 5080-5089, 5090-5099, 5100-5109, 5110-5119, 5120-5129, 5130-5139, 5140-5149, 5150-5159, 5160-5169, 5170-5179, 5180-5189, 5190-5199, 5200-5209, 5210-5219, 5220-5229, 5230-5239, 5240-5249, 5250-5259, 5260-5269, 5270-5279, 5280-5289, 5290-5299, 5300-5309, 5310-5319, 5320-5329, 5330-5339, 5340-5349, 5350-5359, 5360-5369, 5370-5379, 5380-5389, 5390-5399, 5400-5409, 5410-5419, 5420-5429, 5430-5439, 5440-5449, 5450-5459, 5460-5469, 5470-5479, 5480-5489, 5490-5499, 5500-5509, 5510-5519, 5520-5529, 5530-5539, 5540-5549, 5550-5559, 5560-5569, 5570-5579, 5580-5589, 5590-5599, 5600-5609, 5610-5619, 5620-5629, 5630-5639, 5640-5

IASPP 60, completed
IASPP/RAI
IASPP/RAI 2, G.B. corrected T.N.

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IASPP QC translation
IASPP/RAI
IASPP/RAI 2.6 kb converted TLM

THE UNITED STATES DEPARTMENT OF THE INTERIOR
BUREAU OF LAND MANAGEMENT
WASHINGTON, D. C. 20250

ASPP-60 translation
ASPP-RA/
ASPP-RA 12.6 with corrected TTY

AND THE STATE OF CALIFORNIA, COUNTY OF ALBUQUERQUE, do hereby certify that the within and foregoing is a true and correct copy of the original as the same appears from the records of the County of Albuquerque, New Mexico, and the State of California.

IASPP, INC. 4000 Highway 100
LASPP/NOA
LASPP/NOA, Inc. 4000 Highway 100

RESIDUAL EARNINGS LISTING
 360 350 340 330 320 310 300 290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

ASPP ET translation
ASPP/AL
ASPP/BA 256 corrected 77M

BEHAR P. SINGH, CHIEF CLERK, FOR THE P. S. MINISTRY, ON A FIVE YEARS RE-APPOINTMENT, P. S. DEPARTMENT, P. O. OF QUTUB-UL-AZAM-UD-DIN, DISTRICT OF DELHI.

ASPP GC translation
ASPP/HA
ASPP/HA 2.0 kb cotransfected

[illegible]

IASPP-BC Information
IASPP/RAI
IASPP/RAI 2.5 kb corrected T171

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IASPP: EC translation
IASPP/RM/
IASPP/RAL2.6 no corrected TLM

[illegible]

ASP FRAMEWORK USED FOR
LMO-9.3 (NOT FRAMESET)

Fig 3-6



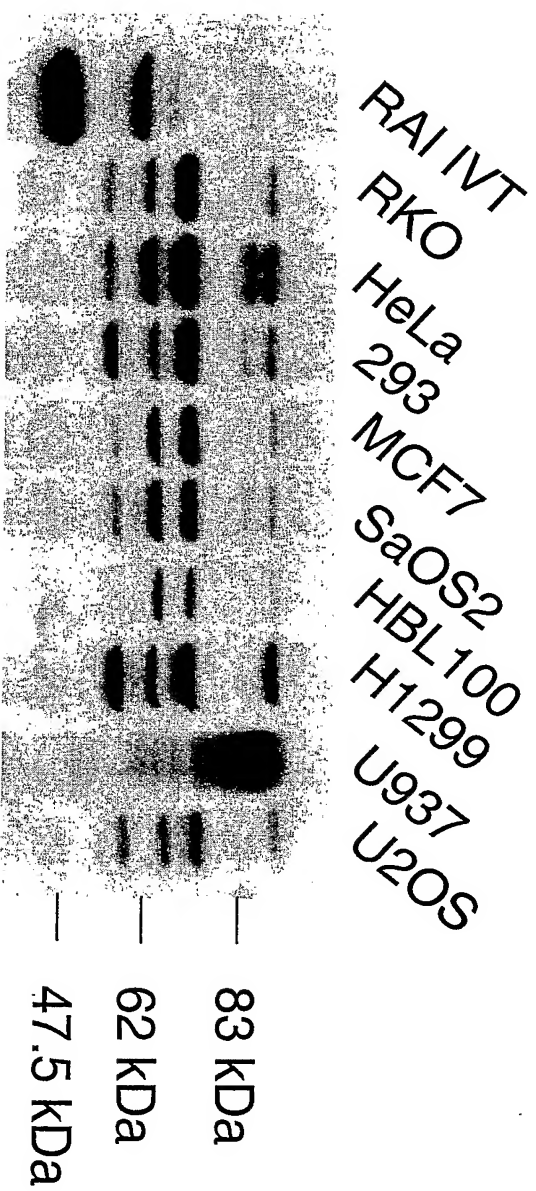
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 670
 680
 690
 700

[illegible][illegible]

Fig 3-7



Expression of iASPP in various cell lines

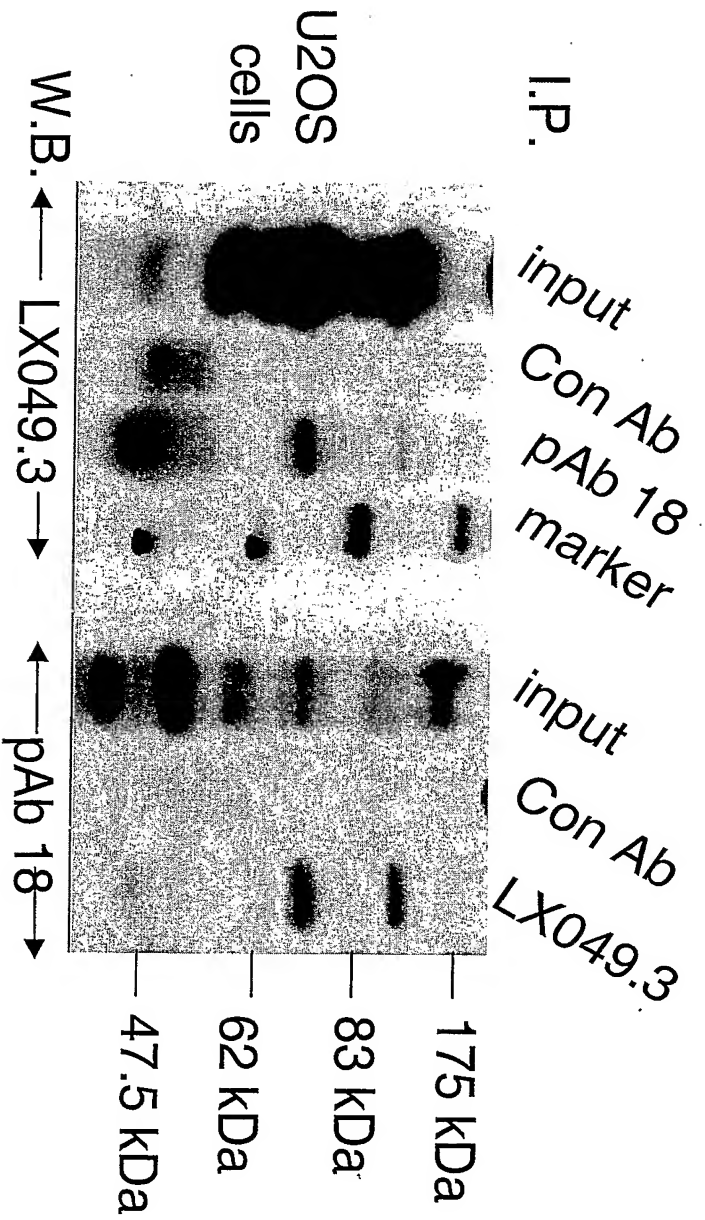


Antibody = LX049.3



iASPP - I.P./Western blot

Fig 5





Effect of cell density and MG132 upon iASPP expression in U2OS cells

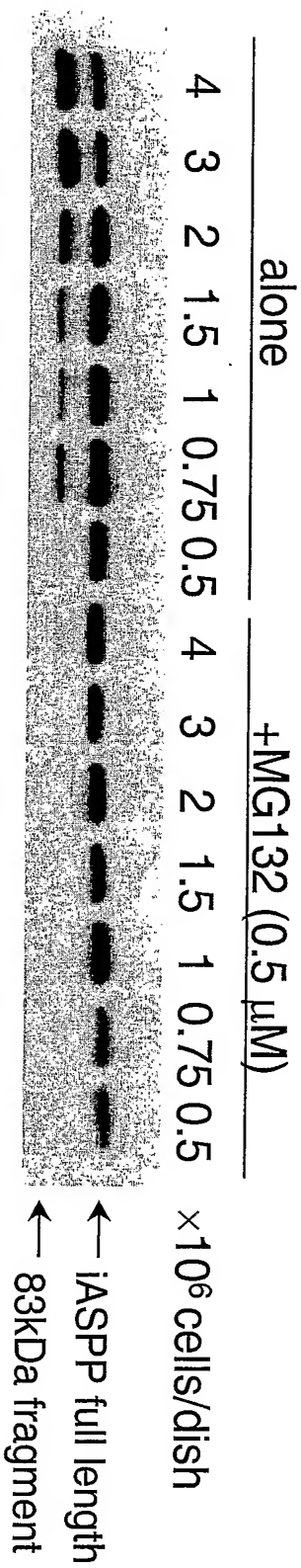
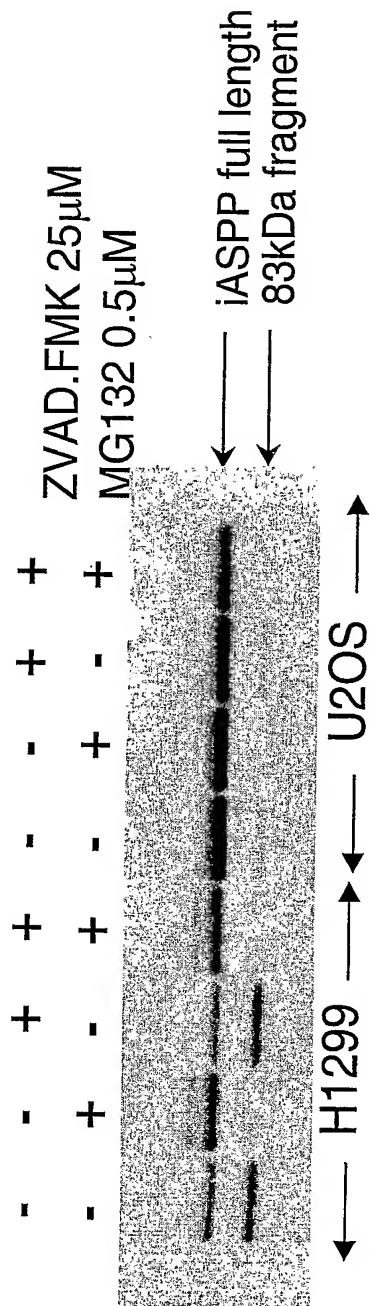


Fig 6a



Fig 6b

Effect of MG132, Z-VAD.FMK upon iASPP



Interaction of iASPP with p53 and Bcl2 in U2OS cells

Fig 7

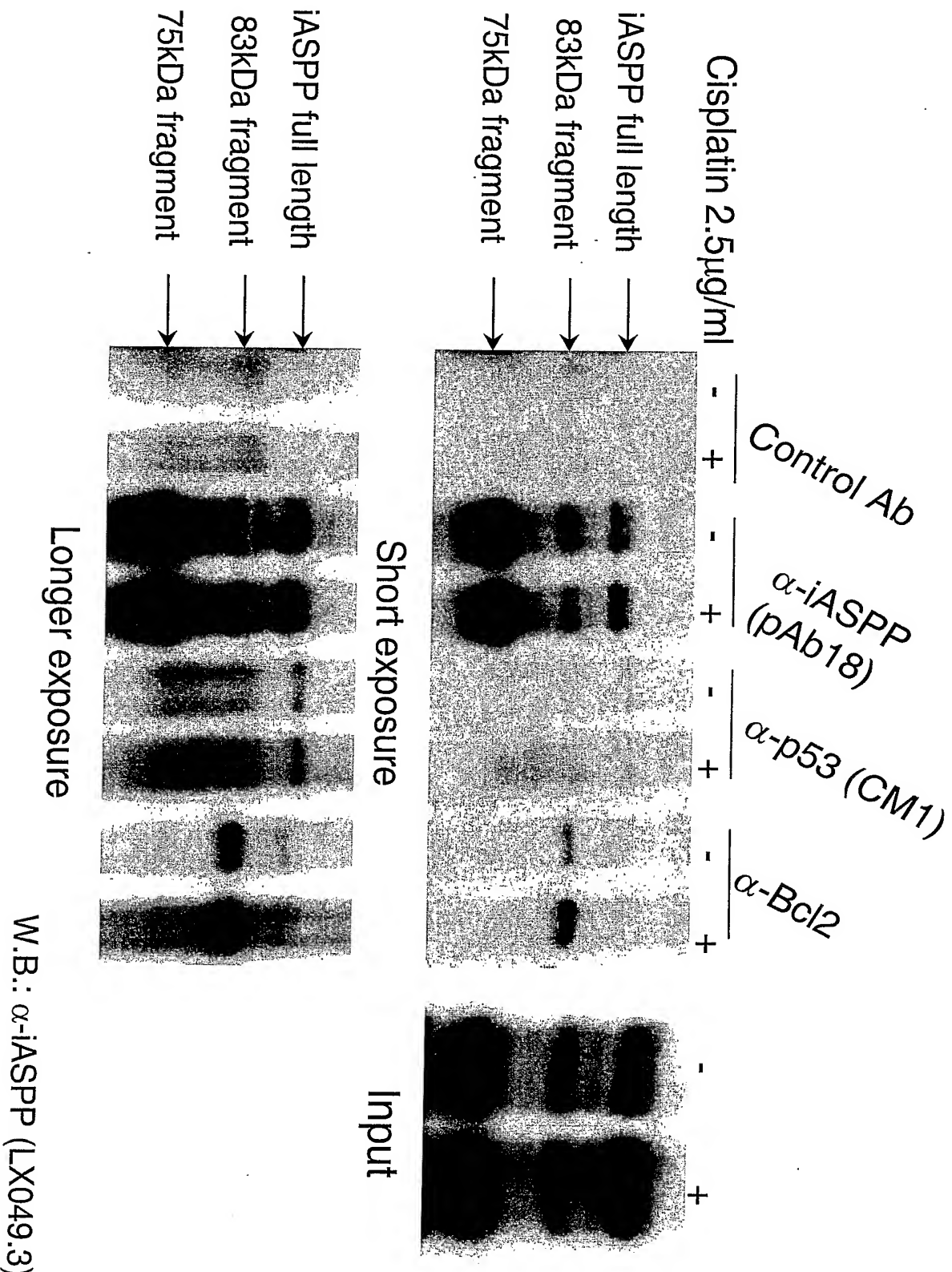
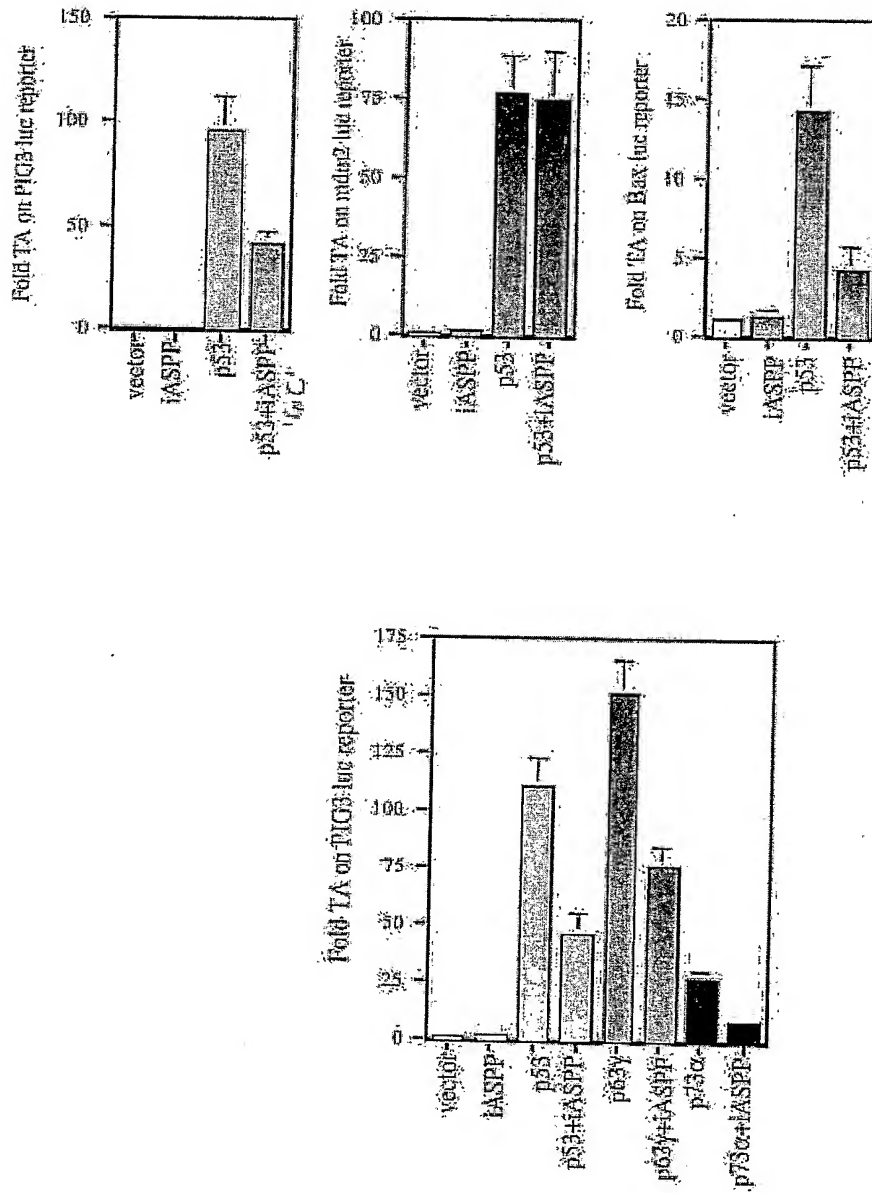
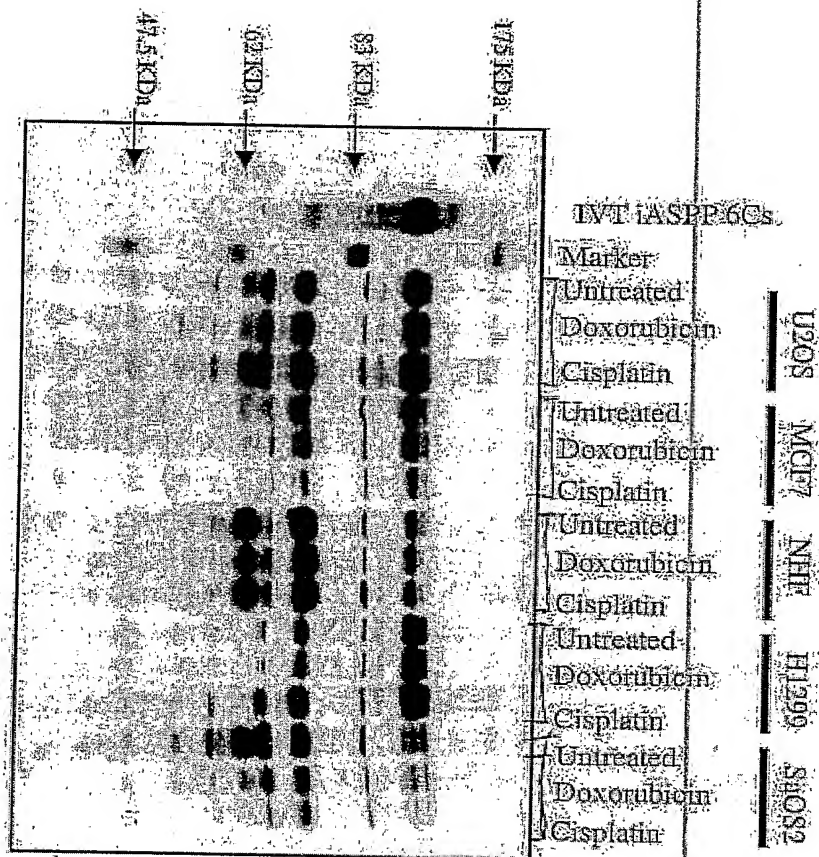


Fig 8

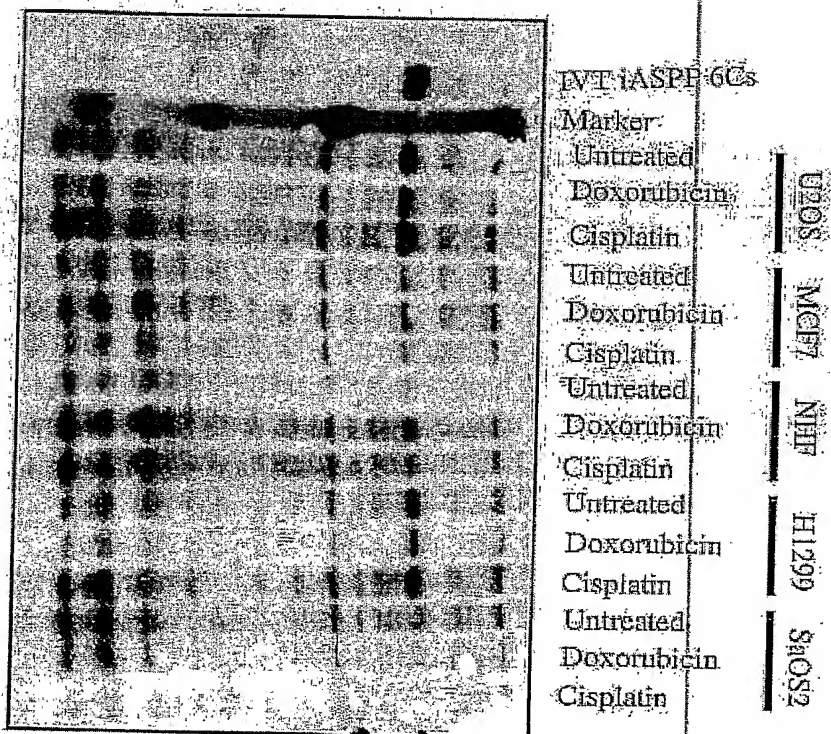


IASPP pattern in five different cell lines

Fig 9



Cell lysates were detected with the monoclonal antibody LX049.3



Cell lysates were detected with the monoclonal antibody S.A.4.2

PCT/GB2004/003492

